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- (54) Title: NOVEL PRKAG3 ALLELES AND USE OF THE SAME AS GENETIC MARKERS FOR REPRODUCTIVE AND MEAT QUALITY TRAITS**

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(57) Abstract: Disclosed herein are genetic markers for animal meat quality and reproductive efficiency, methods for identifying such markers, and methods of screening animals to determine those more likely to produce larger litters and/or better meat quality and preferably selecting those animals for future breeding purposes. The markers are based upon the presence or absence of certain polymorphisms in the PRKAG3 gene.

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What is claimed is:

1. A method of screening animals to determine those more likely to produce larger litters comprising: obtaining a sample of genetic material from said animal; and assaying for the presence of a genotype in said animal which is associated with increased litter size, said genotype characterized by the following: a) a polymorphism in the PRKAG3 gene.
2. The method of claim 1 wherein said polymorphism results in an amino acid change from valine to isoleucine at amino acid number 199 of the PRKAG3 gene or its equivalent as determined by a BLAST comparison of SEQ ID NO:2.
3. The method of claim 1 wherein said polymorphism is a transition of a guanine to an adenine at nucleotide position 595 or its equivalent.
4. The method of claim 1 wherein said genotype is a BsaHI polymorphism.
5. The method of claim 1 wherein said step of assaying is selected from the group consisting of: restriction fragment length polymorphism (RFLP) analysis, minisequencing, MALD-TOF, SINE, heteroduplex analysis, single strand conformational polymorphism (SSCP), denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE).
6. The method of claim 1 wherein said animal is a pig.
7. The method of claim 1 further comprising the step of amplifying the amount of PRKAG3 gene or a portion thereof which contains said polymorphism.
8. The method of claim 7 wherein said amplification includes the steps of: selecting a forward and a reverse sequence primer capable of amplifying a region of the PRKAG3 gene which contains a polymorphic BsaHI site.
9. The method of claim 8 wherein said forward and reverse primers are selected from and based upon primer RNF and primer RNR.

10. A method of screening animals to determine those more likely to exhibit improved meat quality traits comprising: obtaining a biological sample of material from said animal; and assaying for the presence of a genotype in said animal which is associated with improved meat quality traits said genotype characterized by the following:
- 5 a) a polymorphism in the PRKAG3 gene, said polymorphism resulting in and characterized by an amino acid of valine at position 199 and arginine at position 200, or an isoleucine at position 199 when an arginine is at position 200 or its equivalent as determined by a BLAST comparison of SEQ ID NO:2.
- 10 11. The method of claim 10 wherein said polymorphism is a transition of a guanine to an adenine at nucleotide position 595 or its equivalent.
- 15 12. The method of claim 10 wherein said step of assaying comprises a short interspersed element polymorphism test.
13. The method of claim 12 wherein said assay comprises the step of amplifying the PRKAG3 gene using primers selected from and based upon primer RP1F and primer PN52R2.
- 20 14. The method of claim 10 further comprising the step of amplifying the amount of PRKAG3 gene or a portion thereof which contains said polymorphism.
- 25 15. The method of claim 14 wherein said amplification includes the steps of selecting a forward and a reverse sequence primer capable of amplifying a region of the PRKAG3 gene which contains a polymorphic BsaHI site.
- 30 16. The method of claim 14 wherein said forward and reverse primers are selected from and based upon Primer RNF and primer RNR.
17. A method of screening animals to determine those more likely to exhibit improved meat quality traits comprising: obtaining a biological sample of material from said animal; and assaying for the presence of a genotype in said animal which is associated with improved meat quality traits said genotype characterized by the following:
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- 5 a) a polymorphism in the PRKAG3 gene, said polymorphism resulting in and characterized by an amino acid change of asparagine to threonine at amino acid position 30 or its equivalent as determined by a BLAST comparison of SEQ ID NO:1.
- 10 18. The method of claim 17 wherein said polymorphism is a transition of an adenine to cytosine at nucleotide position 89 or its equivalent as determined by a BLAST comparison of SEQ ID NO:1.
- 15 19. The method of claim 17 wherein said genotype is a StyI polymorphism.
- 20 20. The method of claim 17 wherein said step of assaying is selected from the group consisting of: restriction fragment length polymorphism (RFLP) analysis, minisequencing, MALD-TOF, SINE, heteroduplex analysis, single strand conformational polymorphism (SSCP), denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE).
- 25 21. The method of claim 20 wherein said animal is a pig.
- 30 22. The method of claim 20 further comprising the step of amplifying the amount of PRKAG3 gene or a portion thereof which contains said polymorphism.
23. The method of claim 22 wherein said amplification includes the steps of: selecting a forward and a reverse sequence primer capable of amplifying a region of the PRKAG3 gene which contains a polymorphic StyI site.
24. The method of claim 23 wherein said forward and reverse primers are selected from and based upon Primer RF1 and primer RN52R2.
25. A method of screening animals to determine those more likely to exhibit improved meat quality traits comprising: obtaining a biological sample of material from said animal; and assaying for the presence of a genotype in said animal which is associated with improved meat quality traits said genotype characterized by the following:

- a) a polymorphism in the PRKAG3 gene, said polymorphism resulting in and characterized by an amino acid change of glycine to serine at amino acid position 52 or its equivalent as determined by a BLAST comparison of SEQ ID NO:1.

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26. The method of claim 25 wherein said polymorphism is a transition of a guanine to an adenine at nucleotide position 154 or its equivalent as determined by a BLAST comparison of SEQ ID NO:1.

10 27. The method of claim 25 wherein said genotype is a HphI polymorphism.

28. The method of claim 25 wherein said step of assaying is selected from the group consisting of: restriction fragment length polymorphism (RFLP) analysis, minisequencing, MALD-TOF, SINE, heteroduplex analysis, single
15 strand conformational polymorphism (SSCP), denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE).

29. The method of claim 25 wherein said animal is a pig.

20 30. The method of claim 28 further comprising the step of amplifying the amount of PRKAG3 gene or a portion thereof which contains said polymorphism.

31. The method of claim 30 wherein said amplification includes the steps of:
25 selecting a forward and a reverse sequence primer capable of amplifying a region of the PRKAG3 gene which contains a polymorphic HphI site.

32. The method of claim 30 wherein said forward and reverse primers are selected from and based upon Primer RF1 and primer RN52R2

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33. A nucleotide sequence which encodes upon expression an PRKAG3 protein, further comprising a serine at position 52.

34. The nucleotide sequence of claim 33 comprising SEQ ID NO:5.

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35. A PRKAG3 protein according to claim 33.

36. The protein of claim 35 comprising SEQ ID NO:6.
37. A nucleotide sequence which encodes upon expression an PRKAG3 protein, said protein comprising an isoleucine at position 199 and an arginine at position 200 or the equivalent thereof, of said protein.
38. A PRKAG3 protein according to claim 37.
39. A nucleotide sequence which encodes upon expression an PRKAG3 protein, said protein comprising an isoleucine at position 199, a threonine at position 30 a glycine at position 52 and an arginine position 200 or the equivalent thereof, of said protein.
40. A PRKAG3 protein according to claim 39.
41. A nucleotide sequence which encodes upon expression an PRKAG3 protein, said protein comprising a valine at position 199 and an arginine at position 200 or the equivalent thereof, of said protein.
42. A PRKAG3 protein according to claim 41.
43. A nucleotide sequence which encodes upon expression an PRKAG3 protein, said protein comprising an isoleucine or valine at position 199 and an arginine at position 200 or the equivalent thereof, of said protein.
44. A PRKAG3 protein according to claim 43.
45. A method of screening animals to determine those more likely to have favorable meat quality traits comprising: obtaining a sample of genetic material from said animal; and assaying for the presence of a genotype in said animal which is associated with favorable meat quality traits, said genotype characterized by the following: a threonine at amino acid position 30, a glycine at amino acid position 52 and an isoleucine at amino acid position 199.
46. A method of screening animals to determine those more likely to have favorable meat quality traits comprising: obtaining a sample of genetic material from said animal; and assaying for the presence of a genotype in said

animal which is associated with favorable meat quality traits, said genotype characterized by the following: a isoleucine at position 199 and an arginine at position 200.

5 47. A method for identifying a genetic marker for meat quality and/or litter size in animals comprising the steps of: determining the number of offspring produced by each female animal or the meat quality of said animal; determining the polymorphism in the PRKAG3 or equivalent gene of each animal; said polymorphism comprising the polymorphism of claim 1,7,17,or 11 or their
10 equivalents and associating the number of offspring produced by each female animal or meat quality with said polymorphism thereby identifying a polymorphism for animal meat quality or litter size.

15 48. The method of claim 47 further comprising the step of selecting animals for breeding which are predicted to have favorable meat quality or litter size by said marker.

49. The method of claim 48 wherein said analysis comprises digestion of PCR amplified DNA with a restriction enzyme selected from the group
20 consisting of BsaHI, HphI, and StyI.

50. A method for screening animals to determine those with a favorable combination of traits for meat quality and/or litter size, which method comprises of the steps: determining the alleles of PRKAG3 present in a
25 animal said alleles comprising those which include one or more of the following a polymorphic BsaHI, HphI, or StyI site in the PRKAG3 gene; determining the alleles of other markers for genes known to affect meat quality and/or litter size; and selecting for animals with favorable combinations of alleles and against those carrying unfavorable combinations.

30 51. The method of claim 50 wherein the determination of PRKAG3 alleles comprises determining the presence of at least one allele associated with at least one DNA marker linked either directly or indirectly to PRKAG3 .

35 52. The method as claimed in claim 51 wherein the DNA marker is a microsatellite.

53. A method of screening animals to determine those with favorable meat quality traits comprising: obtaining a sample of genetic material from said animal; and assaying for the presence of a genotype in said animal which is associated with favorable meat traits, said genotype characterized by the following:

- a) a polymorphism in the PRKAG3 gene, said polymorphism being one other than the RN- mutation at amino acid 200.

54. A method of screening animals to determine those more likely to have favorable meat quality traits comprising: obtaining a sample of genetic material from said animal; and assaying for the presence of a genotype in said animal which is associated with favorable meat quality, said genotype characterized by a combination of at least two polymorphisms in the PRKAG3.

55. A method of screening animals to determine those more likely to have increased value for litter size and/or meat quality traits comprising: obtaining a sample of genetic material from said animal; and assaying for the presence of a genotype in said animal which is associated with favorable litter size and/or meat quality, said genotype characterized by a combination of at least two polymorphisms in the PRKAG3.

56. A method of screening animals to determine those more likely to have favorable meat quality traits comprising: obtaining a sample of genetic material from said animal; and assaying for the presence of a genotype in said animal which is associated with favorable meat quality traits, said genotype characterized by the following: a threonine at amino acid position 30, a serine at amino acid position 52 and a valine at amino acid position 199.

57. A method of screening animals to determine those more likely to exhibit improved meat quality traits and or larger litter size comprising: obtaining a biological sample of material from said animal; and assaying for the presence of a genotype in said animal which is associated with said traits said genotype characterized by the following:

- a) a short interspersed element polymorphism in the PRKAG3 gene.

58. The method of claim 57 wherein said assay comprises the step of amplifying the PRKAG3 gene using primers selected from and based upon primer RP1F and primer PN52R2.

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